

# Supplementary Information

## Engineering of Gadolinium Decorated Graphene Oxide Nanosheets for Multimodal Bioimaging and Drug Delivery

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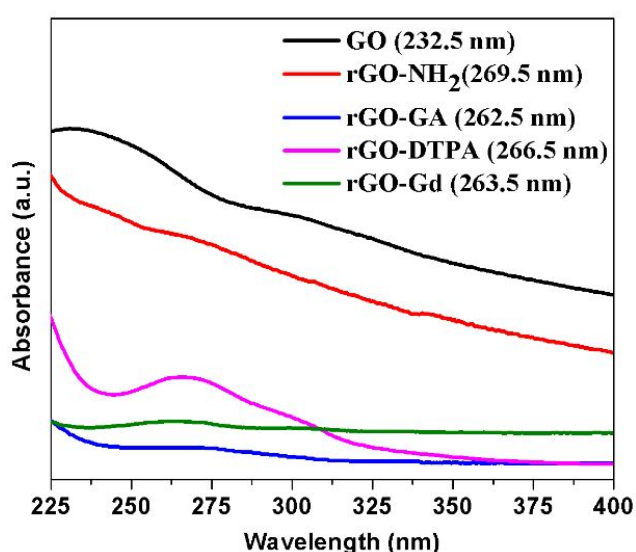
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### 1. UV-Visible analysis

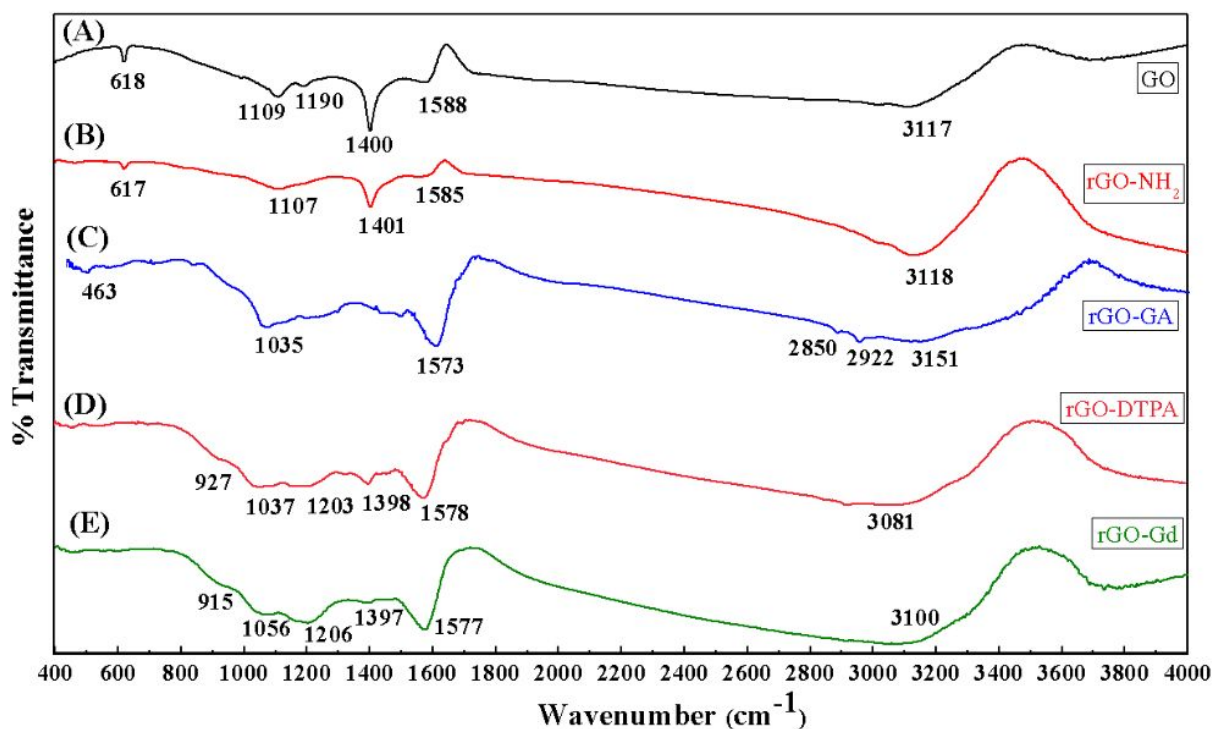


**Figure S1:** Absorption spectra of synthesis steps of graphene oxide to Gd-rGONSs, graphene oxide (GO), reduced graphene oxide-NH<sub>2</sub> (rGO-NH<sub>2</sub>), reduced graphene oxide-gluconic acid (rGO-GA), reduced graphene oxide-diethylenetriaminepentaacetic acid (rGO-DTPA), reduced graphene oxide-gadolinium (rGO-Gd).

Fig. S1. Shows the absorption spectra of all the synthesis and functionalization steps involved from GO to Gd-GO. The absorbance maximum for GO was at ~232 nm whereas for rGO-NH<sub>2</sub>, rGO-GA, rGO-DTPA, rGO-Gd are ~ 269.5 nm, ~ 262.5 nm, ~ 266.5 nm, and ~ 263.5 nm respectively. The red shift of the absorbance peak could be considered due to functionalization which would have caused reduction of GO and restoring sp<sup>2</sup> hybridization type of conjugation<sup>1</sup>. The shift of the absorbance peak is due to conjugation of -NH<sub>2</sub> with a lone pair connected to a  $\pi$  bond. The reduction of GO and p- $\pi$  conjugated effect takes place increasing motion of electrons which cause absorption band to move along higher wavelength<sup>2</sup>.

## 2. FTIR analysis

The demonstrated FTIR spectra show difference in stretching frequency and their shift after each functionalization steps (Fig. S2). Stretching frequencies of GO to Gd-rGO at  $\sim 3117$ ,  $3118$ ,  $3151$ ,  $3081$  and  $3100$   $\text{cm}^{-1}$  is attributed to hydrogen-bonded-O-H stretching vibration.



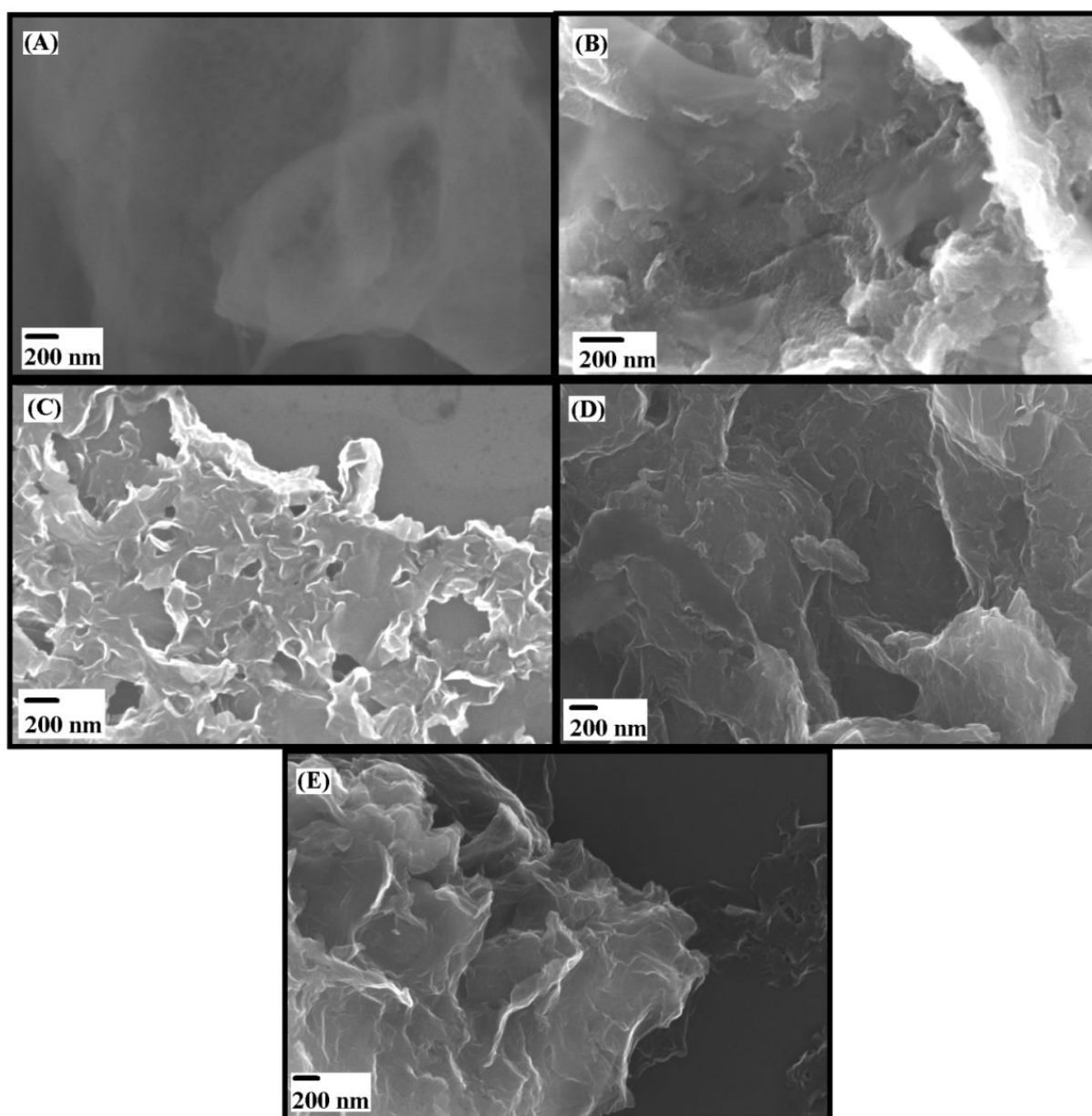
**Figure S2:** Comparative FTIR spectra of graphene oxide (GO-A), reduced graphene oxide-NH<sub>2</sub> (rGO-NH<sub>2</sub>-B), reduced graphene oxide-gluconic acid (rGO-GA-C), reduced graphene oxide-diethylenetriaminepentaacetic acid (rGO-DTPA-D), reduced graphene oxide-gadolinium (rGO-Gd-E).

The obtained spectra of GA-rGO revealed changes over rGO surface after functionalization by organic molecules. This could be observed by stretching frequency at  $\sim 2922$  and  $2850$   $\text{cm}^{-1}$  due to C-H sp<sup>3</sup> stretching. The GO exhibit two minor peaks at  $\sim 1588$  and  $1109$   $\text{cm}^{-1}$  for C=C and C-O-H stretching vibrations respectively. This stretching vibrations were shifted in case of rGO-NH<sub>2</sub> by 3 and 2  $\text{cm}^{-1}$  to  $\sim 1585$  and  $1107$   $\text{cm}^{-1}$  revealing reduction of GO to rGO<sup>3</sup>. Similar change was also observed for 1400 and 1401  $\text{cm}^{-1}$  for O-H bendings. After the functionalization of rGO-NH<sub>2</sub> sample, N-H bending was prominently observed for rGO-GA, DTPA and Gd at 1573, 1578 and 1577  $\text{cm}^{-1}$  respectively. The functionalization of rGO has not brought any deformities over rGO surface as could be evident by slight shift of C-O stretching frequency obtained at 1035, 1037 and 1056  $\text{cm}^{-1}$  for rGO-GA, DTPA and Gd respectively. The similar observation is demonstrated by further functionalization with bulkier molecule like DTPA as two new peak were observed for C=N or C-O deformation

vibration at  $1398\text{ cm}^{-1}$  while C-N or C-O epoxide at  $1203\text{ cm}^{-1}$  respectively for rGO-DTPA. There was slight shift of this stretching frequency to  $1397\text{ cm}^{-1}$  and  $1206\text{ cm}^{-1}$  after decoration with  $\text{Gd}^{3+}$  ions which were chelated by DTPA<sup>4</sup>. The FTIR signature of graphene basal plane is observed in range of  $\sim 600\text{-}900\text{ cm}^{-1}$  which is observed in all the FTIR spectra for samples<sup>5</sup>.

### 3. FESEM Imaging

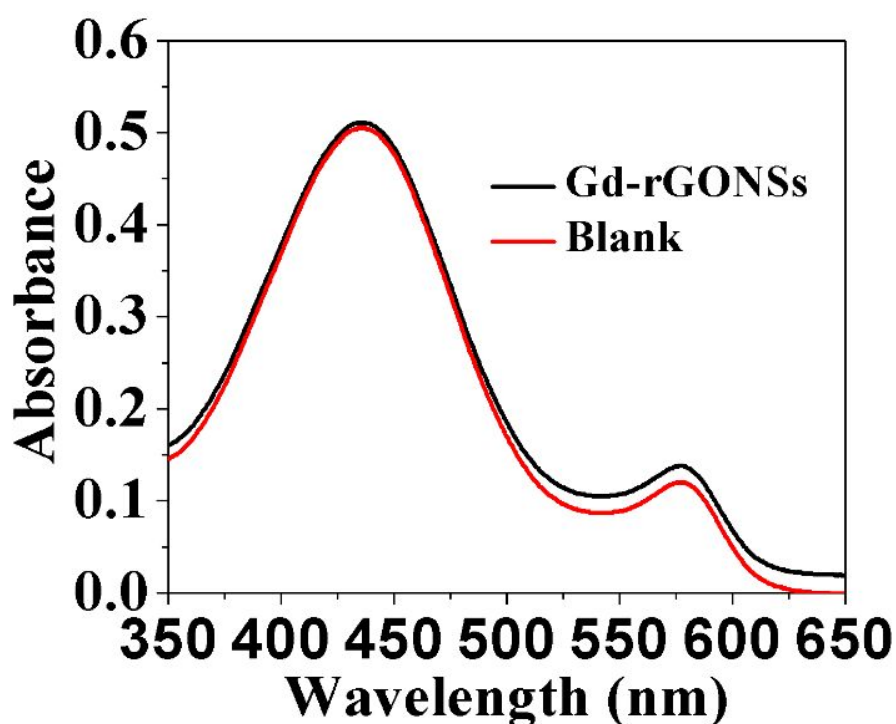
Fig. S3, shows the FESEM images of GO to Gd-rGO, GO showed clear composed structure with only few defects and less wrinkles.



**Figure S3:** FESEM images of synthesis step of graphene oxide to Gd-rGONSs, graphene oxide (GO-A), reduced graphene oxide- $\text{NH}_2$  (rGO- $\text{NH}_2$ -B), reduced graphene oxide-gluconic acid (rGO-GA-C), reduced graphene oxide-diethylenetriaminepentaacetic acid (rGO-DTPA-D), reduced graphene oxide-gadolinium (rGO-Gd-E).

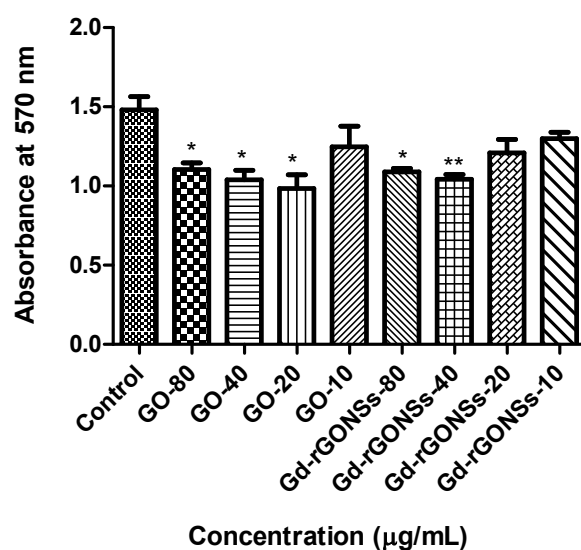
Fig. S2-B, showed different morphology of increased defects which can be found as rich in wrinkles and fluctuation resulted by reduction of the GO surface while changing to rGO-NH<sub>2</sub>. The further functionalization with D-gluconic acid (GA) resulted in fluffy and more transparent morphology. The wrinkles were also reduced which enhanced water dispersity of the rGO-GA sample. Next step of functionalization with DTPA changed morphology by inducing aggregation of the rGO surface and increasing active surface sites (Fig. S3-D). The activated surface was later used for decorating Gd<sup>3+</sup> ions to increase effective surface area and composing surface towards continuous and uniform sheets by reducing fluctuations which is evident from Fig. S3-E.

#### 4. UV-vis spectral of leached Gd<sup>3+</sup> ions in presence of xylenol orange



**Figure S4.** UV-vis spectral of leached Gd<sup>3+</sup> ions in presence of xylenol orange in acetate buffer (pH 5.8) from Gd-rGONSs and compared with blank.

## 5. Cell viability of H1299 cells incubated with GO and Gd-rGONSs



**Figure S5.** Cell viability of H1299 cells incubated with GO and Gd-rGONSs at various concentrations for 24 h. Bars represent means  $\pm$ SEM, n = 3. \* p < 0.05 - \*\*p < 0.01.

## 6. Comparative drug release studies

**Table S1. Comparative drug release studies of 5-FU and Graphene-based Nanocarrier**

| Graphene-based Nanocarrier   | loading/release medium | loading/ release                           | Ref.      |
|--|------------------------|--|-----------|
| Chitosan-functionalized GO   | Water/PBS              | 0.053 mg·mg <sup>-1</sup> / 50%            | 6         |
| ALG functionalized GO  | Water/PBS              | 24.40%/ >60% in the first 3 h              | 7         |
| Magnetic Fe <sub>3</sub> O <sub>4</sub> / GO nanocomposites                          | PBS/PBS                | 0.37 mg·mg <sup>-1</sup> /21% in 24 h      | 8         |
| Graphene nanosheet–CNT–iron oxide NPs hybrid   | Water/PBS              | 0.27 mg mg <sup>-1</sup> /15.7% in 8 h     | 9         |
| Fe <sub>3</sub> O <sub>4</sub> /SiO <sub>2</sub> /graphene–CdTeQDs/CS nanocomposites | pH = 5.5/PBS           | 70 wt%/~90 % in 168 h                      | 10        |
| rGO/AuNCs/SE hydrogel  | Water/PBS              | N.A./~50% in 24 h                          | 11        |
| PVA-SPIONs-GO nanocomposite  | N.A./PBS               | 35.91%/ 80.6%. in 100 h                    | 12        |
| Hollow core/shell Fe <sub>3</sub> O <sub>4</sub> @GO composites                      | PBS/PBS                | 0.41 mg mg <sup>-1</sup> / 11.46% in ~24 h | 13        |
| Gd-GONSs   | Water/PBS              | 0.26 mg·mg <sup>-1</sup> /~92 % in 72 h    | This work |
| GO   | Water/PBS              | 0.19 mg·mg <sup>-1</sup> /~92 % in 72 h    | This work |

**Abbreviations:** GO, Graphene oxide; Gd-GONSs, Gadolinium-Graphene oxide nanosheets; ALG, Sodium alginate; CNT, Carbon nanotube; NPs, Nanoparticles; rGO, reduced graphene oxide; AuNCs, gold nanocages; SE, spinach extract; PVA, polyvinyl alcohol; CS, chitosan; SPIONs, superparamagnetic iron oxide nanoparticles; PBS, phosphate-buffered saline; QDs, Quantum dots; NA, Not Available.

## 7. Comparative $r_1$ relaxivity studies

**Table S2.** Comparative  $r_1$  relaxivity studies of Graphene-based MR contrast agent and Gd-rGONSs

| <b>Graphene-based MR contrast agent</b>  | <b><math>r_1</math><br/>(<math>\text{mM}^{-1} \text{s}^{-1}</math>)</b> | <b>Tesla (T)</b> | <b>Ref.</b> |
|--|---|------------------|-------------|
| GO-DOTA-Gd complex   | 14.2  | 11.7 T           | 14          |
| Poly(amidoamine) dendrimer-grafted gadolinium-functionalized nanographene oxide (Gd-NGO) | 7.59  | 7 T              | 15          |
| Paramagnetic graphene quantum dots (GQDs)-folate-GdGQDs                                  | 11.49   | 7 T              | 16          |
| Gd-GONSs   | 16.85   | 1.5 T            | This work   |

**Abbreviations:** GO, Graphene oxide; Gd-GONSs, Gadolinium-Graphene oxide nanosheets; DOTA, tetraacetate; NGO, nanographene oxide; GQDs, quantum dots.

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